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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/058,323	04/09/1998	BEREND HOUWEN	10690/101683	7347

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EXAMINER
GABEL, GAILENE

ART UNIT	PAPER NUMBER
1641	30

DATE MAILED: 12/30/2002

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/058,323	HOUWEN ET AL.	
	Examiner	Art Unit	
	Gailene R. Gabel	1641	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *Applicant's Response filed 10/30/02* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-13* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-13* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Reopen Prosecution

1. In view of the arguments filed on 10/30/02, PROSECUTION IS HEREBY REOPENED. New grounds of rejection are set forth below.

To avoid abandonment of the application, appellant must exercise one of the following two options:

(1) file a reply under 37 CFR 1.111 (if this Office action is non-final) or a reply under 37 CFR 1.113 (if this Office action is final); or,

(2) request reinstatement of the appeal.

If reinstatement of the appeal is requested, such request must be accompanied by a supplemental appeal brief, but no new amendments, affidavits (37 CFR 1.130, 1.131 or 1.132) or other evidence are permitted. See 37 CFR 1.193(b)(2).

Applicant's Response

2. Applicant's response filed 10/30/02 in Paper No. 30 is acknowledged. Currently, claims 1-13 are pending and are under examination.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the

invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

3. Claims 1-3 and 5-9 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Kim et al. (US 5,648,225) in view of Loken et al. (US 5,047,321).

Kim et al. disclose methods for 1) discriminating and counting erythroblasts, 2) determining classes of leucocytes, and 3) immunophenotyping lymphocytes, using a multipurpose reagent system (see Abstract). The method comprises adding the multipurpose reagent system to an anticoagulated blood sample, incubating the mixture, and subjecting the mixture to flow cytometric analysis (see column 6, lines 6-17). Specifically, the reagent system includes proper concentrations of aldehydes, non-quaternary mono-ammonium salt, and buffer to lyse the nucleated and non-nucleated red cells while maintaining the integrity of the fixed white blood cells (see column 3, lines 60-65). In addition, it includes a buffer that maintains the pH at 5.5-7.5 (see column 7, lines 1-16). Specifically Kim et al. disclose that incubating the blood sample with the reagent system at slightly elevated temperatures, effectively preserves white

cell membrane integrity and retains antigenicity of lymphocyte surface antigens (see column 7, lines 37-41). Certain high concentrations of ingredients to lyse nRBC's are damaging to integrity of white cells and therefore requiring a rapid quenching to the lytic activity of the reagent (see column7, lines 42-47). In addition, the reagent further comprises a nucleotide fluorescent dye, ethidium homodimer, which reacts with exposed nuclei of nRBCs, i.e. erythroblasts, but impenetrable to intact white cells to allow quantitative analysis of nucleated red cells (see column 8, lines 32-54). The reagent also further comprises fluorochrome-conjugated antibodies directed to leucocyte surface antigens to allow quantitative analysis and differentiation of leucocytes, i.e. anti-CD4, anti-CD8 conjugated to FITC, PE, etc. (see column 8, line 65 to column 9, line 22). Electronic signals from scattered light collected from different angles and fluorescence intensities are plotted as two dimensional plots (see column 6, lines 31-46 and also Figure 3).

Kim et al. is silent in teaching that the multipurpose reagent is used in simultaneously analyzing hematological samples to 1) discriminate erythroblasts by detecting nucleotide fluorescent signal and 2) determine leucocyte classes by detecting signal from labeled anti-leucocyte antibodies that bound to cell surface antigens in the leucocytes using multiparameter flow cytometric analysis.

Loken et al. disclose combining a whole blood sample with at least two nucleotide fluorescent dyes (RNA dye or DNA dye) and at least one fluorescent labeled antibody specific for cell surface antigen. The dyes independently and differentially assess different characteristics of nucleated cells in the sample and simultaneously, the

fluorescent labeled antibody specific for cell surface antigens differentially assesses leucocytic cells of different lineages (see column 4, lines 27-40). Each of the dyes and fluorescent labeled antibody, i.e. phycoerythrin (PE) is excitable at the same wavelength and has a peak emission spectra that is distinguishable from the others (see column 5, lines 16-20). The fluorescence intensity and light scatter of the labeled cells in the hematologic mixture is simultaneously measured and analyzed using flow cytometry.

It would have been obvious to one having ordinary skill in the art at the time the invention was made to simultaneously analyze hematological samples using flow cytometry such as taught by Loken, to 1) discriminate erythroblasts by detecting nucleotide fluorescent signal and 2) determine leucocyte classes by detecting signal from labeled anti-leucocyte antibodies that bound to cell surface antigens in the leucocytes such as taught by Kim, since the multipurpose reagent taught by Kim is capable of performing both functions simultaneously by optionally including both of the nucleotide fluorescent dye and labeled anti-leucocyte antibodies in the reagent to differentially stain both populations and Loken specifically taught that multiparametric flow cytometer analysis allows for such simultaneous measurements. Therefore, the presence of both nucleotide fluorescent signal and labeled anti-leucocyte antibodies in the multipurpose reagent taught by Kim, albeit used in discriminating and counting erythroblasts, is capable of discriminating between leucocyte populations as well, or vice versa, using multiparametric flow cytometric analysis measurements as taught by Loken. It has been held that forming in one piece an article which has formerly been formed in two pieces and put together, i.e. multipurpose reagent system having both

nucleotide fluorescent dye and fluorescent labeled anti-leucocyte antibodies, involves only routine skill in the art. *Howard v. Detroit Stove Works*, 150 U.S. 164 (1893).

4. Claims 4 and 10-13 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kim et al. (US 5,648,225) in view of Loken et al. (US 5,047,321) as applied to claims 1-3 and 5-9, and in further view of Inami et al. (US 5,298,426).

Kim et al. and Loken et al. have been discussed supra. Kim et al. and Loken et al. differ from the instant invention in failing to lyse, i.e. permeabilize the cell membranes of RBCs and nRBCs by incorporating reagents and buffers at specific pH and osmolality parameters in a two step method such as set forth in claim 4.

Inami et al. disclose a two-step method of differentiating erythroblasts from leucocytes. Inami et al. specifically disclose mixing blood with a hypotonic fluorescent dye solution capable of diffusing into erythroblasts to stain their nuclei and a buffer for maintaining the pH in the acidic range. Inami et al. further mixes the (acidic) sample mixture with a second fluid comprising a buffer that neutralizes the acidic pH in the solution to a staining pH and an osmolarity adjusting agent for adjusting the osmolarity of the solution to a value at which the shape and integrity of leucocytes are maintained (see column 2, lines 3-24 and column 4, lines 17-41). The first acidic and hypotonic fluid has a low osmolality causing erythrocytic cell lines in the sample to swell upon absorbing water causing cellular contents to leak out and nucleotide fluorescent dye (erythroblastic dye to diffuse through the cell membrane to stain their nuclei. Leucocytes do not permit the entrance of nucleotide fluorescent dye (see column 5, line

60 bridging to column 6, line 26). Inami et al. enumerates the different dyes used in the first fluid for differentiating leucocytes and erythroblasts, including propidium iodide and ethidium bromide specific for erythroblast nuclei, and appropriate concentrations thereof in column 3 of the disclosure. Inami et al. disclose that the concentration of nucleotide fluorescent dye, i.e. propidium iodide or ethidium bromide, should fall within the range of 0.003 mg/L to 10 mg/L (2.5 µg/ml to 100µg/ ml) in order to achieve optimum results (see column 4, lines 5-16). After treatment, stained cells are measured using a flow cytometer and erythroblasts are separated from other cell groups on the resulting two-dimensional plot where erythroblasts are counted (see column 6, lines 9-12). Figure 9 shows a two-dimensional plot showing selective staining of erythroblasts with nucleotide staining dye to emit red fluorescence and to permit erythroblasts to be distributed in a separate zone from other cells so that the relative content and count can be determined. Figure 10 and 11 show two-dimensional plots for the intensity of red fluorescence versus the intensity of side-scattered light obtained for peripheral blood and bone marrow. Inami et al. fail to disclose staining of leucocytes using fluorescent-labeled antibody which specifically binds leucocytes in a hematologic sample.

It would have been obvious to one of ordinary skill in the art at the time of the instant invention to incorporate the teaching of Inami in permeabilizing erythroblasts using reagent combinations having specific pH and osmolality requirements in a two step method, into the reagent system and method disclosed by Kim and modified by Loken because Kim specifically taught that integrity and antigenicity of white blood cells need to be maintained optimally during permeabilization, i.e. lysing, of the nRBC's or

erythroblasts so as to allow accurate simultaneous quantitation of both populations as suggested by Loken, sometimes requiring quenching of lytic activity of the reagent because of its damaging effect to leucocytic populations and Inami specifically taught that such a procedure eliminates such extreme lysing conditions for erythroblasts while maintaining the integrity and shape of WBCs for accurate differentiation of both erythroblast and leucocyte populations.

Kim et al., Loken et al. and Inami are silent in disclosing differentiating between different stages of erythroblast populations. However, Inami specifically disclosed using a same type and concentration of nucleotide fluorescent dye as set forth in claim 10; therefore, it is said that such parameter requirement taught by Inami can effectively perform the same erythroblast maturity differentiation and quantitation as set forth in instant claims 11-13 upon subjecting the sample mixture to flow cytometry .

Response to Arguments

5. Applicant's arguments with respect to claims 1-13 have been considered but are moot in view of the new grounds of rejection.

6. No claims are allowed.

7. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Gailene R. Gabel whose telephone number is (703)

305-0807. The examiner can normally be reached on Monday to Thursday, 6:30 AM - 4:00 PM and alternate Fridays.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Long Le can be reached on (703) 308-3399. The fax phone numbers for the organization where this application or proceeding is assigned are (703) 308-4242 for regular communications and (703) 308-4242 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

Gailene R. Gabel
December 16, 2002

LONG V. LE
SUPERVISORY PATENT EXAMINER
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12/27/02